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RESEARCH ARTICLE

Oxidative Stress Status in Sera, Seminal Plasma and their Correlation with Lead (Pb) and Cadmium (Cd) in Iraqi Infertile Male

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Abstract

Oxidative stress has been identified as a major mediator in various etiologies of male infertility. Lead and cadmium are two of the well-known reproductive toxicants to which human are exposed occupationally and environmentally. These heavy metals can lead to negative effects on the testicular functions. The aim of the current study is to look for the differences in the oxidative stress status in serum and seminal plasma of infertile male patients (oligo, azoospermia) groups compared to that of fertile healthy (control) group and to evaluate lead and cadmium levels in sera and seminal plasma of these groups in comparison to fertile healthy controls. The correlation between their level in seminal plasma and serum was also investigated. The current study included three groups of Iraqi male, 32 infertile patients with oligospermia, with 32 infertile patients with azoospermia, and 37 fertile healthy as a control group. The measured parameters included serum and seminal plasma total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), lead and cadmium concentration. The results in comparison with that of the control groups showed:-

- A highly significant increase differences in serum (TOS), (OSI), Pb, and Cd in serum of all infertile groups and between them.
- A significant decrease in serum TAC of azoospermia group, as well as a significant decrease in serum TAC of oligospermia group with significant differences between these two patients groups.
- No significant differences in seminal plasma TOS, TAC, and OSI in all of the infertile groups.
- In seminal plasma, a highly significant decrease in Cd and Pb of azoospermia group. Meanwhile a highly significant decrease in Pb of oligospermia group with highly significant differences between them.

The results showed no correlation in the measured parameters between serum and seminal plasma of both studied patients.

The Personal correlation analysis of TOS, TAC, OSI, Cd, and Pb revealed the presence of: - in oligospermia group, a strong negative correlation was found between serum, (TOS&TAC), and (TAC&OSI), meantime strong positive correlation between (TOS&OSI) also noticed. In serum azoospermia group a positive correlation between TOS&OSI and negative correlation between TAC &OSI were found. Meanwhile in seminal plasma a negative correlation was found between OSI&TAC. Furthermore, the result revealed, that the non-significant inverse relationship exists between lead and Cadmium concentration in serum and the number of sperm.

Keywords: *Male infertility, Serum & Semen, Total oxidant status, Total antioxidant capacity, Oxidative stress index, lead, Cadmium.*

Introduction

Infertility which defined as the clinical disorder affecting approximately 15% of reproductive-aged couples worldwide. Males are found to be solely responsible for 20-30%

of infertility cases (at least 30 million men worldwide) and contribute to 50% of cases overall [1]. The exposures of numerous environmental agents are linked with fertility disorders in human populations [2].Exposure to lead (Pb) and cadmium (Cd) occurs through the air, drinking water, diet, and ingestion of dirt and paint chips. These metals have been shown to be associated with an overproduction of reactive oxygen species and an impairment of anti-oxidant defensive capacity [3].

Cadmium and lead are not widespread in the environment, but they have been extensively used in industry. They are persistent in the environment once discharged, and when absorbed they stay in the animal and human body with long half-lives (long biological half-life 10-30 years in humans). These behavioral characteristics make them good long-term markers of environmental pollution. Several studies have been reported in the literature review deals with the effect of heavy metals in serum and semen in infertile male such as [4, 10].

Many studies resemble to the current study deal with the association of lead, Cd with oxidative stress such as [11, 13]. Lately, oxidative stress has become the focus of interest as a potential cause of male infertility. ROS are products of normal cellular metabolism within the mitochondria [14].The sperm cells are very rich in mitochondria because they constantly need an energy supply to maintain their motility [3].During the enzymatic tetravalent reduction of oxygen to water, energy is produced, but oxygen free radicals, in particular superoxide anion (O^{-2}), are formed as by-products [15].

Reactive oxygen species represent a broad category of molecules including radical (hydroxyl ion, superoxide, peroxyl, etc.), and non-radical (ozone, singlet oxygen, lipid peroxide, hydrogen peroxide), oxygen derivatives [16]. Reactive nitrogen species (nitrous oxide, peroxynitrite, nitroxyl ion, etc.) are nitrogen free radicals and are considered as a subclass of ROS [17].

Since ROS has both physiological and pathological roles, an array of antioxidants maintains a steady state of ROS in the seminal plasma. These antioxidants are compounds which scavenge the formation or oppose of the actions ROS. Normally, equilibrium exists between reactive oxygen species (ROS) production and antioxidant scavenging activities in the male reproductive organs to prevent the damaging effect of excessive ROS, enzymatic and nonenzymatic antioxidant pathways scavenge excess ROS and allow a balance to be achieved between beneficial oxidant generations and damaging harmful ROS [18, 19]. The oxidative stress (OS) which is the result of an imbalance between the formation of reactive oxygen species (ROS) and the inability of the available antioxidants to neutralize the excessive production of ROS and results in lipid peroxidation, protein changes and DNA damage and sperm death [20].

Generally, spermatozoa are particularly susceptible to ROS because of inherent deficiencies in intracellular antioxidant enzyme protection, thus total body antioxidant capacity become more substantial to protect sperm [21].High amounts of poly unsaturated fatty acid are found in the mammalian spermatozoa membranes, making them susceptible to lipid peroxidation and reactive oxygen species (ROS) play major roles in reproduction, they are strongly associated with oxidative stress [22].

Several studies that focused on the role of oxidative stress and antioxidants in male infertility have been reported in the literature review they have reported that free radicals and reactive oxygen species play major roles in reproduction such as [21, 24].The purpose of the current study was to quantify cadmium, lead and oxidative status in fertile and infertile Iraqi males, such measurement enables an assessment the effect of the exposure to such pollutants on the oxidative status and infertility through the correlation of these values with seminal quality.

Materials and Methods

Samples

Serum and semen samples were collected from 101 male at Kamal –Alsamerae hospital, including 70 infertile patients with age ranged (22-50) years compared to 40 fertile males as a control with age range compatible to that of the patients. The patients were evaluated by full medical

history to exclude any existing systemic diseases that may affect the study parameters (hormonal disease, diabetes, hypertensive, liver disease, renal and cardiac disease).The patients received no antibiotic treatment within one week and were non-smoker and non-alcoholic, otherwise the patient was excluded from this study. The study groups were classified depending on seminal fluid analysis according to the WHO criteria (2010) [25] into three groups:-

- Fertile individuals (control group) (n=37) sperm count > 20 million /ml, progressive motility > 50%.
- Infertile individuals (oligospermia) (n=32) sperm count < 20 million/ml, progressive motility <40% and sperm morphology >50%.
- Infertile individuals (azoospermia) (n=32) sperm count 0%. No sperm in ejaculate.

The protocol of this study was approved by the ethics committee of College of Science/University of Baghdad.

Semen Analysis

Semen analysis was determined according to WHO criteria25.The semen samples were collected from all study male individuals (101 male) who had been abstinent for 3 to 5 days before the semen collection, liquefaction of semen occurs after a period of 30 min. Semen quality was evaluated macroscopically within one hour of collection by using microscopic examination. The routine seminological analysis was carried out estimating sperm density, motility, viability and morphology.

Blood Samples

Five millilitres of venous blood samples were collected by using disposable needle and plastic syringes from each patient and control. The samples were transferred into clean plain tube, and was left at room temperature for 15 min then separated by centrifugation at (3000 x g) for 10 minutes. The obtained serum was stored at (-20) ºC until being used for subsequent analysis.

Measurement of Total Oxidant Status [TOS]

This status was determined in serum & seminal plasma samples according to the method described by [26].The result were measured by using a colorimetric method and expressed as (µmol H_2O_2 Eq. /L).

Determination of Total Antioxidant Capacity [TAC]

Total antioxidant capacity was determined in blood serum & seminal plasma according to the method described by [26].

Calculation of Oxidative Stress Index (OSI)

Oxidative stress index value was calculated from the following equation [27].

OSI =TOS (µmol H_2O_2 Eq./L)/ TAC (µmol uric acid Eq./L)

Determination of Lead& Cadmium Concentration

The lead and cadmium concentration in serum and seminal plasma were measured using Flame atomic absorption spectrometry with air-acetylene flame and hollow cathode lamp. The used wavelengths for lead measurement were 283.2 nm while for cadmium was 228.9 nm [28].

Statistical Analysis

Statistics was performed with SPSS (version 23) program. The results were expressed as mean± SD .The statistical test used were, one way a nova test.

The correlation coefficient(r) between parameters was determined by analyzing for linear regression, multiple range test at $p<0.05$ was accepted as significant, highly significant when $p<0.001$, and the range test at ($p>0.05$) is accepted as non-significant.

Results and Discussion

Human are exposed to various environmental agents that may be hazardous to their reproductive capacity. The toxic effect of heavy metals can also be a factor in male infertility [22]. The concentration of cadmium and lead in blood serum and seminal plasma

of the present study groups were determined and the results are as shown in Table (1).

Table 1: Mean values of cadmium and lead concentration in the serum and seminal plasma of control and patients groups

Parameters	Control N=37 (32.97±8.25)	Oligospermia N=28 33.97±8.09)(	Azoospermia N=26 32.7±9.9)(
Serum cadmium conc µg/ml)(	0.073±0.019	0.127±0.045 a ** p value :0.035 b p value :0.000 a	0.145±0.022 a **b* p value :0.000 a
Seminal plasma cadmium conc(µg/ml)	0.141±0.028	0.134±0.020 p value :0.000 b p value :0.294 a	0.089±0.032 a **b** p value :0.000 a
Serum Lead conc. (mg/ L)	0.168±0.056	0.813±0.379 a** p value :0.000 b p value :0.000 a	1.250±0.200 a **b** p value :0.000 a
Seminal plasma Lead conc. (mg/ L)	0.830±0.213	0.693 ±0.131 a** p value :0.000 b p value :0.000 a	0.045±0.020 a**b** p value :0.000 a

**The difference is a highly significant at the 0.001 level. *The difference is a significant at the 0.05 level
A refers to difference in comparison to control: b refers to difference between two patients group

It is obvious from these results there are highly significant increase ($p<0.001$) in serum cadmium concentration in oligospermia and azoospermia groups compared to that of their control group, with significant variations ($p<0.05$) between two patients groups. Meanwhile, a highly significant decrease ($p<0.001$) in seminal plasma cadmium and lead of azoospermia group is noticed. Moreover, a highly significant decrease in seminal plasma lead level of oligospermia group as compared to the control group with highly significant variation between two patients group (oligo, azoospermia) are observed. Also it is clear from the above table that there is a highly significant increase($p<0.001$) in serum lead concentration of oligospermia and azoospermia group compared to that of their control group, while in seminal plasma a highly significant reduction ($p<0.0001$) is observed and a highly significant variation between two patients group (oligo, azoospermia) is present.

The observed markedly elevated levels of Cd and Pb in serum were observed in the present study agree with many studies such as the study of Xu *et al*. in China who studied the trace elements in blood and seminal plasma and their relation to sperm quality [29]. Meanwhile the study of Akinolye *et al*. in Nigeria who found a significant increase in serum cadmium level of infertile groups as compared with the fertile group [17]. As well as consistent with the findings of Riaz *et al*. in Pakistan who study the association between heavy metals and stress marker [13]. Meanwhile a study of Hassani *et al* in Iraq who study the effect of heavy metal pollution and men infertility in Al-Falluja city [9].Also agree with the study of (Famurewa *et al* in Nigeria who aimed to evaluate association of blood and seminal plasma lead and cadmium with sperm quality of non-occupationally exposed male partners of infertile couples with infertility and found high levels lead and cadmium in infertile groups as compared with the fertile group, while disagree with their results on semen where they found adverse effect [30].The results of low Cd, Pb levels in seminal plasma of infertile (oligo & azoospermia) groups compared with control group, agree with the results presented by Hovalta *et al*. who worked on lead and cadmium concentration in seminal plasma in Finnish men, the study revealed low levels of Cd and lead in the spermatozoa, suggesting that the low exposure may be one explanation for the relatively good sperm quality [4].

Another study result agrees with the low concentration of Pb in seminal plasma observed in the present study, their data revealed that the lead concentration in semen did not induce oxidative DNA damage in human sperm and this may be due to the concentration of lead of their patient compared to that of fertile one [31]. On the other hand, the results of low concentration of lead and cadmium in semen in the present study disagree with many of studies such as a study of Pant *et al.* in India who found high lead and cadmium concentration in their semen samples [6]. Also the study of (Taha *et al*. in Egypt who study the correlation between seminal lead and cadmium and seminal parameters in idiopathic infertile male and found higher levels of these elements in their semen and suggested that this differences may be due to the multitude of influencing geographical differences including environmental and life style[12]. To consider the status of oxidative stress, it is generally necessary to estimate the antioxidant parameters and the oxidant markers such is TAC, TOS, and OSI which are recommended to be done in normal and pathological status. Table (2) shows the mean value of oxidative stress parameters (TOS, TAC, OSI) and the concentration of Cd and lead in the serum and seminal plasma of control and patients groups (oligo, azoospermia).

Table 2: Mean values± SD of oxidative stress parameters in the serum and seminal plasma of all study groups

Parameters	Control Age(32.37±8.25) N=37	Oligo Age(33.97±8.09) N=32	Azoo Age(32.7±9.09) N=32
TOS serum (µmol H_2O_2 Eq/l)	7.60±0.68	9.40±0.93 p value 0.000a**	8.52±0.92 p. value 0.000a**b**
TOS seminal plasma (µmol H_2O_2 Eq/l)	5.26±0.67	5.36±0.75 p value 0.21a	5.58±0.73 p value 0.06a 0.16b
TAC serum (mmol Uric acid Eq/l)	5.34±0.67	4.56±0.77 p value 0.000a**	5.05±0.61 p value 0.008a* 0.005 b*
TAC seminal plasma (mmol Uric acid Eq/l)	2.10±0.31	2.03±0.44 p value 0.53 a	2.01±0.55 p value 0.41a 0.86b
OSI serum	1.42±0.20	2.08±0.37 p value 0.000a**	1.68±0.27 p value 0.000a**b**
OSI seminal plasma	2.53±0.45	2.64±0.66 p value 0.41a	2.79±0.70 p value 0.06a 0.22b

**The difference is a highly significant at the 0.001 level. *The difference is a significant at the 0.05 level
A refers to difference in comparison to control: b refers to difference between two patients group

The results show presence of highly significant increase ($p< 0.001$) in serum TOS for the two patients groups when compared to the control group. Meantime highly significant differences ($p<0.001$) between the two patients groups (oligo & azoospermia) were observed. While in seminal plasma, no significant variation were found in both patients compared with control and between them. The findings of both patients groups (oligo & azoospermia) in serum TOS agrees with many studies in different countries that measured individual oxidative stress parameters, and showed that there was an oxidative stress in sera of their patient's groups. Such as a study by in Iraq, which reported an increase in malondialdehyde (MDA) in sera of their patients' groups [32], and verify the findings of many studies such as [13, 33, 34].As well as a study in Jordan that their results revealed highly increase of ROS production in sera of patients' groups [35].

In seminal plasma, the TOS results agree with the study of N'guessan *et al*.,2016 in Turkey, who found no significant differences in (MDA) in infertile men in comparison with that of the fertile one [36]. Meantime disagree with the results of AL-Khyatt *et al.* & Riaz *et al*. who reported the presence of a significant elevation in TOS semen in their infertile groups when compared with the fertile group [32, 33]. As well as the study of Salimi *et al*. who have described higher level of ROS by measuring (MDA) of varicocele infertile men compared with fertile men [37]. The observed increase in serum TOS of infertile groups (oligo & azoospermia) may be due to many causes such as increased level of ROS production and lipid peroxidation by the abnormal spermatozoa. The elevation of ROS damages the blood-testis barrier, reaches the

tests and ultimately become the part of seminal plasma [13]. The oxidative stress (OS) is characterized by an overabundance of ROS, or deficiency in antioxidants. ROS are products of normal cellular metabolism and causes damage at the molecular level, which impairs lipid, protein, and DNA [38].

This elevation may be due to increased exposure to heavy metals such as lead and cadmium which their levels indicated in (Table 1) of the present study. These metals have been shown to be associated with an over production of reactive oxygen species and an impairment of anti-oxidant defensive capacity [16].Since cadmium is a non-redox metal, it is unlikely to participate in Fenton type reaction, furthermore, Cd can compete with essential metals in protein binding sites leading to the release of Fe^{+2} and Cu^{+2} ion causing increased production of ROS and oxidative stress, it was reported that an elevated concentration of Cd has a toxic effect on iron-dependent enzymes such as cytochrome P450 [7]. Lead has two common valences, $Pb^{+2}$and Pb^{4} and does not by itself catalyzes free radical reaction. On the other hand lead on its Pb^{+2} state can apparently enter cells by passing through Ca^{+2}channels and combines quickly with-SH groups on proteins and at high concentration, can cause GSH depletion [11] thus lead can cause metabolic ,enzymatic,oxidative,and genetic damage by producing adverse effects on the body's organs [39]. Antioxidants are the most important defense substances against free radical induced infertility [40].

Human body provides a complete antioxidant defense system composed of enzymatic and non-enzymatic antioxidants molecules [16]. Total antioxidant capacity (TAC), which reflects the total effect of all antioxidants existing in a body fluid, was measured in serum and seminal plasma and the results are presented in Table (2). These results showed that there is a highly significant decrease in TAC in the serum of oligospermia group ($p<0.001$), a significant decrease ($p<0.05$) in that of azoospermia group compared with the control group, significant differences ($p<0.05$) between the two patients groups. Meanwhile, there are no significant differences in this parameter among seminal plasma of both patients groups ($p>0.05$).

The obtained results in serum agree with many studies in different countries and with the result of measurement of individuals oxidative stress parameters, which showed that there was a decrease in antioxidants parameters in their patient's groups [32, 35, 41], and disagree with the study of (Giulini *et al* in Italy ,who found no significant differences in blood TAC concentration between control and patients groups while their seminal plasma TAC were significantly lower in patients groups than control [13], and disagree with the result of Benedetti *et al* in seminal plasma TAC who reported significant decrease in the patients groups compared with the control group [41]. The possible explanation for the decreased TAC in serum may be due to the fact that sperm motility is essential for normal fertilization and requires normal healthy progressive motile sperm to fertilize ovum, defect in sperm motility is common cause in infertile men [42]. During passage through the epididymis the spermatozoa acquire motility and the progressive movement requires the presence of a specific epididymal protein called the forward motility protein [43]. Spermatozoa protect itself via a complete antioxidant defense system composed of enzymatic and non-enzymatic antioxidants. Because of the lack of the intracellular antioxidant enzymes sperm cannot protect the plasma membrane that surrounds the tail, forcing to supplement their limited intrinsic antioxidant defenses by depending on the seminal plasma protection, thus make sperm tail depends on the entire body antioxidant capacity protection [21].

The proposed mechanism for loss of sperm function may be due to excessive generation of ROS [44] and the observed non-significant decrease may be due to the reported decrease of uric acid as it is a main contributor to the chain-breaking antioxidant capacity of blood and seminal plasma [34].Low antioxidant levels induce oxidative state which destroys the forward motility protein by its oxidation & this may cause the decrease in the progressive motility [43]. Also low antioxidant levels cause destruction of the tail membrane by ROS since spermatozoa depend on the antioxidant capacity of seminal plasma to protect itself and the low serum antioxidant levels decrease seminal plasma antioxidant capacity.

It was suggested that glutathione and protein thiols protect lipids in the sperm cell membrane from oxidation and prevents the

formation of free oxygen due to the fact that thiol group is a strong nucleophile and provide protection against damage by electrophilic oxidants [45]. One factor that was reported to duplet glutathione (in its reduced form) and protein bound sulfhydryl groups, which act as important antioxidants defense system is cadmium [46]. Such reduction results in enhanced production of free radicals such as superoxide ion, hydroxyl radical and hydrogen peroxide. The OSI may be a more accurate index of oxidative stress in the body because it is a comprehensive measurement of TAC and TOS which is calculated as the ratio of TOS to TAC. When the oxidative stress index was calculated for sera and seminal plasma of both patient groups and the results are presented in Table (2). The results show there is a highly significant difference ($p<0.001$) in sera OSI of both patients groups (oligo & azoospermia) compared to its control group and between them. Meanwhile, in seminal plasma, no significant differences were observed in all studied groups ($p>0.05$). The observed increase in OSI in the serum of both patients groups (oligo & azoospermia) is due to the low levels of antioxidants present in these groups. The serum Cd levels in the infertile groups were higher than that of controls.

There is also a corresponding significant decrease in seminal plasma Cd of infertile groups, since the testes are among the principal target sites for cadmium; it is likely that cadmium elicits its toxic effect, probably expressed as infertility. Since the metabolism of Cd is similar to Zn metabolism and cadmium has molecular homology with zinc and calcium compensates with them for resorption to the body, the potential for Cd to reduce Zn level and generate reactive oxygen species may be exacerbated, leading to alteration in the antioxidant defense system and imposing oxidative stress and lipid peroxidation in spermatozoa so low level of zinc may cause cadmium shift from blood to seminal plasma compartment or vice versa as these findings were confirmed in the present study by the observed elevation of total oxidative stress (TOS), decrease antioxidant capacity(TAC) with low level of zinc (results under publication) in sera samples of infertile male.

Although cadmium can indirectly increase levels of ROS and induce oxidative stress (OS) leading to lipid peroxidation, DNA damage, and cell death by binding to sulfhydryl groups of the ROS regulators, such as glutathione. Such decrease in the ability of sulfhydryl molecules to scavenge ROS [47], explain the decrease in sulfhydryl oxidase concentration measured in sera and seminal plasma of oligospermia group when compared with that of the fertile one in the present study (results under publication). Furthermore the significant variation in the results between oligospermia and azoospermia group observed in the present study may be due to the lack of sperm number and motility in azoospermia as compared with that of the oligospermia one. Also in the current study, the measured serum Pb level was higher in infertile groups compared with the control, while in semen the adverse effect was found. The biological role of Pb in male infertility is still unclear. Animal studies have reported that lead can affects spermatogenesis and reduces number of spermatozoa within the epididymis in mice administered lead, and resists spermatogenesis in rats [48]. However, similar data in humans is generally limited, lead detrimental spermatogenesis rather than lead altered hypothalamic pituitary gonadal function may be responsible for the association between seminal lead concentration and sperm count.

Lead has been shown to contribute in alteration in the sperm chromatin condensation which has been associated with a low percentage of fertilization& the effect of Pb on sperm nucleus in the epididymis by binding to nuclear sulfhydryl groups from the DNA protamine complex in the epididymis delays nuclear decondensation [12], which might be the cause for fertilization failures observed after lead exposures. Meanwhile, in Australia a study of [48] who showed that the blood-testis barrier can protect testicular cells from direct exposure to high levels of blood lead. For these reasons and considering the wide spectrum of lead toxicity on reproductive hormones, the study suggested that lead's main influence on male reproduction probably occurs by altering the reproductive hormonal axis and the hormonal control on spermatogenesis, rather than by a direct toxic effect on the seminiferous tubules of the testes.

Finally, the presence of trace metals may also alter the effects of heavy metal exposure. Furthermore, diet, particularly intake of

calcium, iron, and antioxidants can modify the effect of a given metal exposure on health endpoints including reproductive ones [15, 49]. Pearson correlation was applied and the result revealed that non-significant inverse relationship exists between lead and Cadmium concentration in serum and the number of sperm (data has not been shown for non-significant value). The present study agrees with Omu *et al.* who reported non-significant association of Cd with semen parameters in fertile and infertile groups·

Meanwhile the result of lead correlation with sperm parameters agrees with the findings of [30, 48].

The average values of the biochemical parameters in serum and seminal plasma have been shown in Figure (1 a, b) respectively.

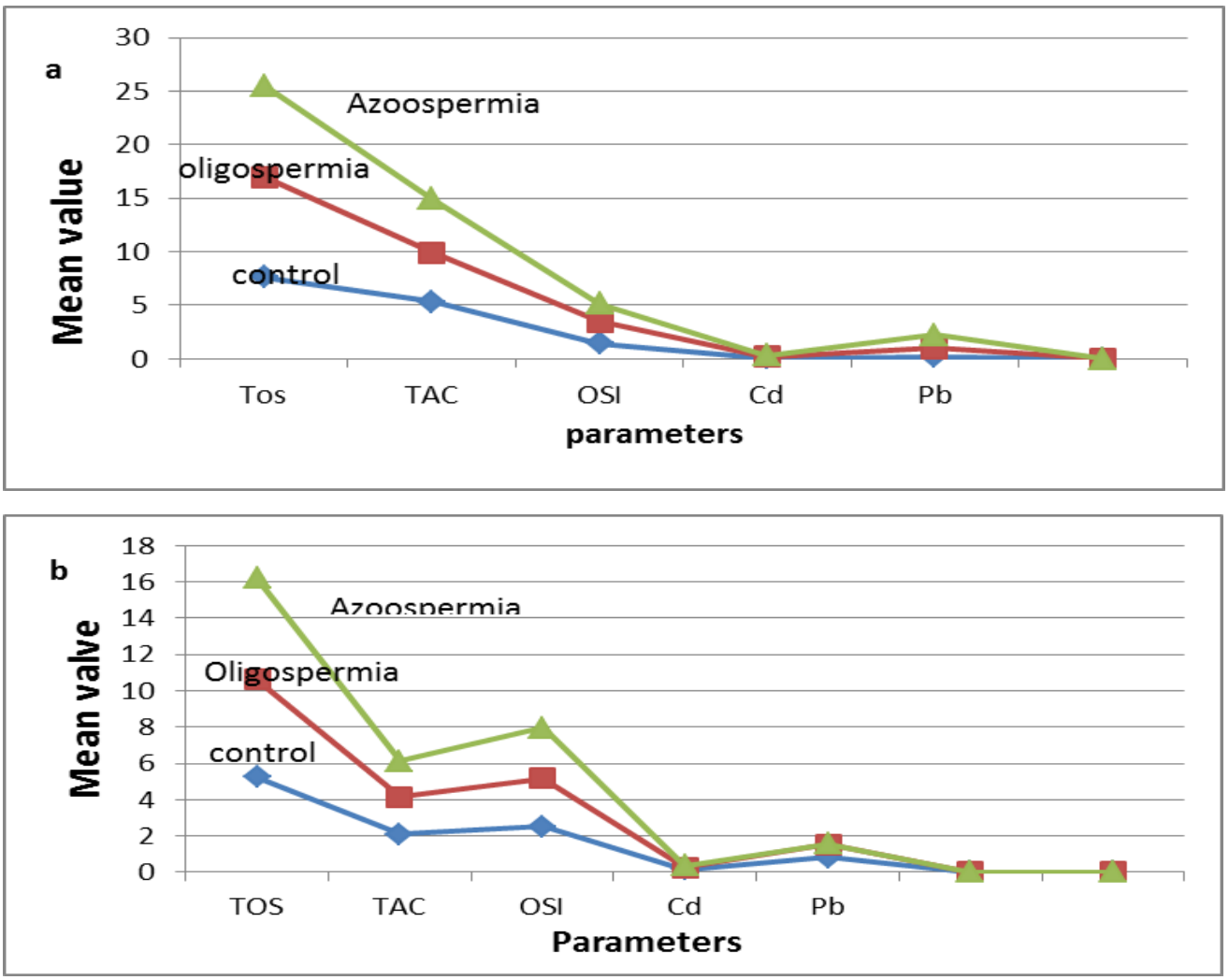

Figure 1; a, b: All average values of biochemical parameters in the serum and seminal plasma of fertile and infertile (oligo, azoo) groups respectively

In serum, Among the infertile groups, the levels of TOS ,OSI Pb, and Cd are significantly high compared to the control group were observed as shown in Figure(1a). Meanwhile in seminal plasma the level of Cd and Pb were significantly lower in azoospermia group than the other groups Figure (1b). The personal correlation was applied to assess the relationship among studied parameters and to check the possibility of using semen as a sample of analysis instead of serum .There is no significant correlation between serum and seminal plasma of all the parameters (data has not shown for non-significant correlation), meanwhile Table (3) showed the significant correlation among all parameters included in the present study.

Table 3: Pearsonal correlation between all the parameters of all the studied groups

Association between	Pearson Correlation (r)
TOS and TAC in serum of control group	0.74**-
TOS and OSI in serum of control group	1.000**
OSI and TAC in serum of control group	-0.74**
TAC and OSI in seminal plasma of control group	-0.72**

TOS &TAC in serum of oligospermia group	0.88**-
TOS&OSI in serum of oligospermia group	1.000**
TAC &OSI in serum of oligospermia group	0.80**-
TOS&OSI in serum of azoospermia group	0.58*
TAC&OSI in serum of azoospermia group	0.74*-
OSI&TAC in seminal plasma of azoospermia group	0.68*-

**Correlation is a highly significant at the 0.001 level. – Negative correlation
*Correlation is significant at the 0.05 level

Conclusions

The obtained data revealed that the study of such parameters in blood plasma cannot be used as an indicator of what happening in seminal plasma .Meanwhile the marked elevation in serum (TOS), (OSI), Pb, and Cd of both the infertile groups and between them were noted. Furthermore, Cd and Pb were negatively correlated with sperm number within non-significant value.

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